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GRANT NUMBER: DAMD17-94-J-4164

TITLE: Psychophysiological Reactions and Immunological Sensitivity to Stress in Healthy Women at Familial Risk for Breast Cancer

PRINCIPAL INVESTIGATOR: Doctor Sandra G. Zakowski
Doctor Dana H. Bovbjerg

CONTRACTING ORGANIZATION: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

REPORT DATE: January 1997

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	January 1997	Final (1 Jan 95 - 31 Dec 96)

4. TITLE AND SUBTITLE
Psychophysiological Reactions and Immunological Sensitivity to Stress in Healthy Women at Familial Risk for Breast Cancer

5. FUNDING NUMBERS
DAMD17-94-J-4164

6. AUTHOR(S)
Doctor Sandra G. Zakowski
Doctor Dana H. Bovbjerg

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Sloan-Kettering Institute for
Cancer Research
New York, New York 10021

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

To date 99 women with and without family histories of breast cancer have been recruited. Of those, 84 women have participated in the experimental assessment, 24 women with family histories and 58 women without family histories of breast cancer. Subjects are exposed to two stressful tasks in the laboratory and psychobiological reactivity is measured in response to these tasks. We report several findings: 1) Women with family histories of breast cancer have greater psychobiological reactivity to acute stressors than women without family histories of cancer. This suggests that these women not only experience chronic distress due to the threat of cancer but also show greater stress responses to an acute event. 2) Positive and negative mood changes contribute to heightened immunological sensitivity to the stressors. 3) Hostility and depression are associated with higher natural killer cell activity at baseline. These last two findings show the importance of subjective moods in determining immune function and has methodological implications for PNI research in cancer.

14. SUBJECT TERMS Breast Cancer	15. NUMBER OF PAGES 19
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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1/6/97

PI - Signature Date

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Introduction

Chronic and acute stress have been associated with alterations in immune measures including Natural Killer (NK) cell activity (e.g., Herbert & Cohen, 1993). Healthy individuals with family histories of cancers have been shown to have lowered NK cell cytotoxicity (e.g., Strayer et al., 1984, 1986; Hersey et al., 1979). As NK cells are thought to serve an important function in immune surveillance against neoplastic cells (Trinchieri, 1990) it is possible that deficits in NK cell activity in individuals at familial risk for cancer may contribute to their heightened risk of developing the disease. It therefore becomes important to determine the causes of this lowered NK cell activity. Aside from heritable deficits in NK cell activity it is also possible that the higher levels of distress that have been found in women with family histories of cancer (e.g., Kash et al., 1992) may be partly responsible for their immune deficits. The present study explores the possiblity that women with family histories of breast cancer may have higher psychophysiological reactivity and/or greater immunological reactivity to stress than women without family histories of cancer. This aim was addressed using an experimental stressor paradigm that has been widely recognized in psychophysiological and psychoneuroimmunological research.

Having a family history of breast cancer is one of the most significant risk factors for developing breast cancer in one's lifetime (Harris, et al., 1992). The risk that a healthy woman will develop breast cancer increases with the number of affected individuals in her family. For example, the relative risk of breast cancer for a woman with a single first degree relative with breast cancer has been estimated as 1.5 to 2; with two first degree relatives estimates range from 4 to 6 relative risk.

In addition to the role played by heritable mutations in specific preneoplastic cell types in familial cancer, it is also possible that heritable deficits in natural killer cell activity could contribute to the increased risk (Strayer et al., 1984; Strayer et al., 1986; Hersey et al., 1979; Guanti et al., 1989; Whiteside et al., 1989; Lotzova, 1991). Natural killer cell activity has been cited as a principal effector mechanism of immune surveillance against transformed cells by a number of investigators (Trinchieri, 1990). Consistent with possible heritable immune deficits, several studies (although not all [Pross et al., 1984]) have found reductions in NK cell activity in individuals with a family history of a variety of different types of cancer. Strayer and colleagues (1984), for example, showed that healthy women with a family history of breast cancer had lower NK cell activity than women without family history. The mechanisms responsible for the reduced NK cell activity have not been explored. These studies, however, raise the possibility that heritable deficits in NK cell activity may contribute to the risk of breast cancer in women with family histories of breast cancer.

Psychoneuroimmunology (PNI) research suggests an additional mechanism by which women with family histories of breast cancer may have lowered NK cell activity, namely the possibility that stress may contribute to this deficit. This idea is especially compelling since these women have been found to have higher levels of both cancer-specific and general distress

than women without such family histories. This has been demonstrated in several studies (Valdimarsdottir et al., 1995; Zakowski et al., in press). The reasons for this higher distress may be numerous, including exposure to a greater number of chronic stressors such as the experience of the death of a parent from cancer, or high levels of perceived risk for breast cancer (e.g., Zakowski et al., in press).

There are at least two ways in which stress may contribute to the reduced immune function seen in this population. The first concerns potential heightened chronic stress due to the greater number or severity of life stressors encountered in individuals with familial histories of cancer. This possibility is currently being investigated in a longitudinal study. The second possibility is that this population may be more sensitive to existing stressors by showing greater psychophysiological and immunological reactions to stressors. The examination of this hypothesis was the principal aim of the present experimental study.

Naturalistic studies have consistently found altered immune function in humans facing a variety of distressing life events ("stressors") including: living close to Three-Mile Island following the nuclear accident (Baum et al., 1983; McKinnon et al., 1989); surviving the death of a spouse (Bartrop et al., 1977; Schleifer et al., 1983; Irwin et al., 1987); enduring marital problems and divorce (Kiecolt-Glaser et al., 1987; Kiecolt-Glaser et al., 1988); taking care of sick relatives (Kiecolt-Glaser et al., 1987; Kiecolt-Glaser et al., 1991); being unemployed (Arnetz et al., 1987); and taking academic examinations (Dorian et al., 1982; Halvorsen et al., 1987; Glaser et al., 1992). The most consistently reported immunological changes in these studies are reductions of *in vitro* measures of cell-mediated immune function (e.g., proliferative responses to T cell mitogens and natural killer (NK) cell function) (Herbert et al., 1993; Biondi et al., 1987). This observation is directly relevant to the stress-health relationship since an altered immune system may not be efficient in protecting the body from the pathogens that can cause illness and may facilitate development of infectious illnesses or reduce the body's ability to reject tumors.

In addition, laboratory studies have demonstrated that experimental procedures designed to induce psychological distress also cause changes in immune function (Manuck et al., 1991; Zakowski et al., 1992; Kiecolt-Glaser et al., 1992; Stone et al., 1993; Kiecolt-Glaser et al., 1988; Zakowski et al., 1994; Zakowski, et al., 1996; Sieber et al., 1992). In these studies human subjects are exposed to standardized acute stressors in a controlled laboratory setting and their psychological and physiological responses are measured over time. This methodology has been widely used in cardiovascular research to measure reactivity to stressors, i.e., the magnitude of the psychophysiological changes from resting baseline levels. Several studies have shown that brief exposure to a relatively mild stressor such as mental arithmetic, Stroop, cold pressor task, aversive noise, or watching a combat surgery film can induce rapid and transient changes in various measures of cellular immunity including increases in NK cell activity (e.g., Delahanty et al., 1997) and lymphocyte proliferation (e.g., Manuck et al., 1991; Zakowski et al., 1992). These immune changes occur within minutes of exposure to the stressor. Aside from eliminating confounds associated with correlational studies this experimental approach also facilitates the study of

individual differences in reactivity, the magnitude of subjects' psychophysiological and immune responses.

The present study examined whether women at familial risk for breast cancer may be psychophysiologically more reactive to stressors than women without family histories of cancer which in turn may affect their immune system. The possibility of higher reactivity in these women is consistent with two published research findings. First, individuals with high levels of life stress have been reported to be more reactive to brief laboratory stressors (Pardine et al., 1983). Second, increased emotional reactivity was reported by Pettingale et al. (1985) in a study of individuals with cancer, a difference which could have predated the development of their cancer. In that study, women with breast cancer reported more distress after watching stressful movies than a group of healthy comparison women (Pettingale et al., 1985).

In addition, individuals with a family history of breast cancer may be more immunologically reactive to stress. That is, their immune system may respond with greater magnitude to a given stressor than that of individuals at normal risk for cancer. This may be due to the higher levels of distress that they may be experiencing in the face of a given stressor, or it may be independent of their level of psychological distress suggesting that any given level of distress might have a greater impact on immune function in these individuals. The idea of an increased immunologic reactivity to psychological distress has been raised by other investigators concerning other populations with compromised immune function, such as the elderly and HIV positive individuals (Ader et al., 1991). The present study examined whether women with family histories of breast cancer may be more immunologically reactive to stressors.

The present study thus examined two major hypotheses: 1. Whether women with family histories of breast cancer may be more psychophysiologically reactive to an acute experimental stressor; and 2. Whether these women may be more immunologically reactive to acute stressors. This was accomplished by comparing women at familial risk for breast cancer to women who are at normal risk based on their family histories. As part of a broader, still ongoing research study, these groups were exposed to acute experimental stressors in the laboratory and their psychological, cardiovascular, and immunological responses were assessed over time.

Method

Subjects

Two groups of women were recruited for this study. The first, women with family histories of breast cancer (FH Group, n=24), i.e., who had at least one first-degree relative with the disease, were recruited from two cancer surveillance clinics in the New York metropolitan area, and advertisements in the community. The second group consisted of women who have no family history of cancer in their first and second degree relatives (NFH Group; n=58). These were

recruited through advertisements in the community. Subjects were included in the study if they were premenopausal, not on birth control pills in the last two months, reported having regular menses, had no history of chronic disease or neoplasm, no history of abnormal breast biopsy or mammogram, and were not currently using any drugs known to affect the immune system.

Procedures

All experimental sessions were started in the morning between 8 and 9 am. Upon arrival at the laboratory, subjects were seated in a comfortable chair, the procedures were explained in detail, and informed consent was obtained. An iv-catheter was then inserted into a vein of the non-dominant arm. A normal saline drip kept the line patent for the duration of the experimental session. The blood pressure monitor was hooked up and the cuff put on the subject's dominant arm. The subject was then allowed to rest for 20 minutes to allow recovery from the blood drawing procedures. Following the rest period, three baseline blood pressure (BP) and heart rate (HR) measures were taken and a blood sample for baseline immune measures was withdrawn. Subjects were then exposed to two consecutive mental tasks or a non-stressful control task. Self-reported distress, cardiovascular, and immunological measures were assessed before and after stressor exposure. After task completion, subjects were told to relax and read magazines for an additional 90 minutes during which time blood samples were drawn at 15 to 30 minute intervals (see below). Upon completion of this session, subjects were debriefed and thanked and were paid for their participation.

Experimental tasks. Both stress tasks were administered by a second experimenter previously unknown to the subject. This experimenter was blind to the subject's family history status. The first stress task consisted of a five-minute speech task, where subjects were asked to imagine they had been caught for a traffic violation and had to defend themselves in traffic court. They were given two minutes to prepare the speech and three minutes to deliver it in front of a video camera in the presence of the experimenter. It was emphasized that their performance would later be rated for content and style and on how convincing their arguments were. The second task was a five-minute mental arithmetic task with harrassment where subjects were told to add numbers out loud at the pace set by an audiotape. The difficulty of this task lies in its demands of speed and concentration on the part of the subject. The subject is presented with a series of one-digit numbers and she is required to add each new number to the previous number given by the tape. It is the tendency of most subjects, however, to add the new number to the solution they gave to the previous number addition because that is the most salient stimulus at that time. Throughout the task, the experimenter interjected comments suggesting to the subject that they needed to work harder or faster.

A non-stressful control task was administered to a subsample of the normal risk women. This served as a manipulation check of the effectiveness of the stress tasks in inducing psychological and immunological changes. The control task consisted of a non-stressful reading

task which subjects completed in the same amount of time. They were asked to read a passage of a text that was provided to them by the experimenter for two five-minute intervals. Results of this manipulation check will be presented separately below.

Measures

1. Background measures:

Demographic questionnaire. This is a standard questionnaire that assesses basic demographics such as age, race, education, marital status, etc.

Family History Questionnaire: Subjects are asked to provide information on family history of cancer, including number of relatives who have had cancer, the type of cancer they had, their age at diagnosis, the outcome of their illness. The information from this questionnaire was used to calculate familial risk for breast cancer according to the Claus Model (Claus, et al., 1994).

2. Measures of acute stress in response to the laboratory manipulations: Reactivity to the laboratory stressors was assessed at several levels. Psychological distress was assessed by self-report questionnaires, cardiovascular reactivity was assessed by continual monitoring of heart rate (HR) and blood pressure (BP)

The Profile of Mood States (POMS). The POMS (McNair et al., 1971) was used to assess levels of distress that subjects experienced at baseline and during the tasks. This is measured using 65 items which are rated on a 5-point Likert type scale. The questionnaire yields six mood subscales including tension-anxiety, depression-dejection, anger-hostility, confusion-bewilderment, vigor-activity, and fatigue-inertia. The POMS also includes a total mood disturbance (TMD) score created by the taking the mean of the scores on all of the items (McNair et al., 1971). Subjects were asked how they felt "right now" at baseline and how they felt "during the tasks" immediately post-task.

Visual Analog Scales (VASs). VASs (Cella et al., 1986) were used to provide measures of subjects' distress and moods at baseline and in response to the stressors. Subjects were asked to indicate on 100mm lines to what extent they experienced each of ten moods, including fatigue, anxiety, confusion, depression, energy, anger, tension, relaxation, frustration, and nervousness. Subjects were asked to rate how they were feeling "right now" at baseline and how they felt "during the tasks" immediately after each five-minute task.

Cardiovascular measures. Blood pressure (BP) and heart rate (HR) were monitored before (baseline) and during the tasks at 2-minute intervals using an automated monitoring device (Spacelabs). This was done by placing an inflatable cuff on the subject's arm at the beginning of the session. Approximately three initial measures were taken prior to the actual baseline

measures, in order to allow the subject to habituate to the device.

3. Immune measures: A total of six blood samples were taken during each experimental session: At baseline (pre-task), 15 min (immediately post-task), 30 min, 45 min, 75 min, and 105 min. The immune measure of primary interest is NK cell activity, because of the published data indicating deficits in individuals with a family history of cancer (e.g., Strayer et al., 1984). In addition, psychoimmune studies have repeatedly documented that NK cell activity is sensitive to emotional distress (Herbert & Cohen, 1993).

Natural killer cell activity. Natural killer cell activity was assessed in a whole blood chromium release assay using the natural killer (NK) cell sensitive K562 erythroleukemia line according to published methods (Rees & Platts, 1983). Whole blood was collected in heparinized vacutainers and stored on ice until processed. All samples were processed on the day of the experiment (within three hours of blood collection). K562 target cells were suspended in 0.2 ml RPMI-NBCS and labelled with $Na_2\ ^{51}CrO_2$ (100uCi) for 1 hour at 37°C. Cells were washed 3 times and resuspended in RPMI-NBCS for one hour. Cells were then washed and resuspended at 1 x 10^5 cell/ml of RPMI/NBCS. Whole blood samples were prepared in four dilutions of whole blood to RPMI-NBCS, i.e., 1:1, 1:2, 1:4, and 1:8. Of each of these dilutions, 0.1 ml was dispensed in round-bottom microtest plates and 0.1 ml of the K562 target cell suspension was added to each well. Each whole blood sample was tested in triplicates at each of the dilutions. Control wells contained 0.1 ml K562 cell suspension with 0.1 ml RPMI-NBCS to assess spontaneous release, or with 0.1 ml detergent to assess maximum release. Well plates were incubated for 4 hours in a humidified incubator at 37 °C and 5% CO_2. Supernatants of each well are then harvested and counted in a gamma-counter. Results will be expressed in percent cytotoxicity.

Results

Manipulation check

First, we examined the effectiveness of the experimental tasks in inducing immune changes by comparing the stress vs. control groups on NKCA percent changes from baseline. A comparison of the two groups using a repeated measures ANOVA on NKCA showed that there was a significant change over time in response to the stressor, $F(1,64)=6.47$, $p<.01$, with an increase of about 32% at the 15 minute timepoint (see Figure 1). This is consistent with previous literature. As expected, moods as measured by the POMS also showed significant changes in response to the stressor (all p's<.05). Using the POMS total mood disturbance (TMD) score and subscales as predictors in a repeated measures regression in the subjects exposed to the stressors, it was shown that mood changes contributed significantly to NKCA changes in response to the tasks. The TMD, positive and negative mood change were predictive of change in NKCA in response to stress, $F(1,45)=4.04$, $p<.05$, $F(1,45)=6.33$, $p<.01$, $F(1,45)=3.51$, $p=.06$, respectively.

Mood subscales, including hostility and confusion were also predictive of NKCA changes, $F(1,45)=4.07$, $p=.04$, $F(1,45)=5.23$, $p<.02$, respectively. This confirms that NKCA is sensitive to negative moods induced by the experimental tasks that were chosen for this study.

Insert Figure 1 about here

Having confirmed the effectiveness of the experimental tasks by the above manipulation checks, we then proceeded to test the main hypotheses of this study by testing the differential psychobiological and immunological reactivity in high risk vs. normal risk women.

Risk group comparability

Subjects included in this part of the analyses were those for whom complete family history data that allowed calculation of breast cancer risk as well as complete immune and psychophysiological data were available at the time of analyses. Women were divided into high risk (n=16) and normal risk (n=30) groups according to their scores on the Claus Model using a cutoff score of 11% (Claus et al., 1994). This classification was made independently of initial family history status, as this model takes into account additional factors (e.g., second degree relatives with cancer, etc.) that make it a more sensitive measure of familial breast cancer risk (Claus et al., 1994). Initial analyses were conducted to ascertain demographic comparability of the two groups. Subjects did not differ significantly on any of the demographic measures, including age (M=34.4, SD=6.8; M=36.25, SD=7.2 for high and normal risk women respectively), marital status (82% of the women were not currently married), education (89.13% had at least a college education. For ethnic background the distributions was as follows: In the normal risk group 66.67% were Caucasian, 13.67 African American, 6.67% Hispanic, 6.67% Asian, and 3.33% other. In the high risk group, 93.75% were Caucasian and 6.25% other.

Psychophysiological reactivity:

Repeated measures ANCOVA was conducted on each of the dependent variables separately including self-reported distress, BP, and HR (see Table 1). Results showed significant differences between the groups on negative moods with the high risk group reporting significantly greater negative mood increases in response to the stressors than women in the normal risk group, $F(1,44)=7.66$, $p<.01$. Heart rate reactivity was also significantly greater in high risk women as shown by greater levels of change from baseline in response to the stressor, $F(1,44)=3.95$, $p=.053$. No group differences were found for blood pressure.

Insert Table 1 about here

Immunological reactivity to stress

Repeated measures ANOVA was conducted using a 4 (dilutions) by 2 (groups) design on percent changes of NKCA lysis from baseline levels. As the initial manipulation check showed the crucial timepoint for stress-NKCA effects to be immediately post-task, we chose this timepoint for the present analysis. Results indicated a significant group main effect indicating that women in the high risk group exhibited greater increases in NKCA in response to the stressors than women in the low risk group, $F(1,44)=8.22$, $p<.01$ (see Table 2). There was also the expected main effect for dilutions confirming a dose-response curve, $F(3,132)=18.56$, $p<.01$. No interactions of group by dilution were found suggesting that the group effect is consistent across NK cell dilutions.

Insert Table 2 about here

Discussion and Conclusions

The present study had two major aims: (1) to examine whether women with family histories of breast cancer have higher psychophysiological reactivity to acute stressors, and (2) whether these women may have greater immunological reactivity to acute stressors. Both of these hypotheses were at least partially confirmed in that it was shown that women at familial risk for breast cancer exhibited greater increases in negative moods and HR, as well as NKCA. Using stress reactivity in the laboratory as a model for responses in everyday life, this may be two of the ways in which women at familial risk for breast cancer may have reduced basal levels of NKCA. First, their heightened psychological and cardiovascular reactivity can be considered to be reflective of high levels of sympathetic arousal that women at familial risk may experience in the face of everyday stressors. This possibly repeated hyperresponsiveness may in turn lead to chronically lowered immune function. Further, it was found that women at familial risk for breast cancer had higher immunological responsivity to acute stressors, suggesting that their immune systems may be more sensitive to stress as well. Whether this is entirely dependent on the extent of their experience of distress in response to the event or whether it may be partially independent of psychological and/or physiological stress responses remains to be examined. It is possible that women at familial risk for breast cancer, in addition to experiencing more distress, also may have immune systems that are more sensitive to this distress. This possibility will be examined in further analyses.

The study has several implications. First, it suggests the possibility that women with family

histories of breast cancer in addition to having higher levels of chronic distress as previously shown (e.g., Valdimarsdottir et al., 1996) because of possible exposure to more stressful events such as the death of a family member from cancer (Zakowski et al., in press), also may be more psychophysiologically reactive to any given stressor. This in turn may contribute to their heightened levels of chronic distress. Future studies should be done to examine the generalizability of this finding to other acute stressors and to stressors in daily life. Further, it should be examined whether this heightened reactivity may be even more pronounced in response to cancer-specific stressors as compared to general acute stressors such as the ones examined in the present study. If it can be shown that women at familial risk for breast cancer are generally more reactive to stressors, interventions can be tailored to this specific issue. For example, teaching women how to reduce their psychophysiological responses to stressors, i.e., through relaxation, meditation exercises, or other techniques may help women to monitor their level of stress responding and reduce their reactivity to a more tolerable level. Future examination of women's reactivity to different types of stressors, i.e., cancer-specific vs. general stressors, may also provide important information in terms of targetting different situations in which stress reducing strategies may be especially valuable and by helping to make the environment more predictable and controllable for these women. Reducing stress responding in women at familial risk for breast cancer may in turn increase their natural killer cell activity which has been shown to be reduced relatively to women without family histories of cancer.

The finding of increased immune responsivity to stressors in these women needs to be further explored. As mentioned earlier, an increase in NKCA is the typical acute stress response found in human experimental studies, therefore a higher increase in NKCA would signify a greater stress response. It is possible that the temporary increase in NKCA may lead to delayed reductions in NKCA that were not picked up in the relatively short time period covered by the experimental sessions. However, this possibility requires further study. In addition, the possibility of greater immunological sensitivity in women at familial risk for cancer, i.e., greater immune responses to stressors when changes in psychological distress are controlled, will also be explored in the present study.

Future studies should be done to confirm our findings and to extend them by examining women's responses to different types of stressors including cancer-specific ones, and examining the effects of interventions targeting women's hyperresponsiveness to stressors by teaching stress-reducing techniques. The consequences of such interventions for immune function need to be addressed as a means for increasing NKCA to levels comparable to those of women without family histories of cancer as a possible way of improving health and reducing cancer risk.

References

Ader R, Felten DL, Cohen N. (1991). Psychoneuroimmunology, Academic Press, San Diego, California

Arnetz BB, Wasserman J, Petrini B, Brenner SO, Levi L, Eneroth P, et al. Immune function in unemployed women. Psychosomatic Medicine 1987; 49(1):3-12

Bartrop RW, Lockhurst E, Lazarus L, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. Lancet 1977; 1:834-836.

Baum A, Gatchel RJ, Schaeffer MA. Emotional, behavioral, and physiological effects of chronic stress at Three Mile Island. Journal of Consulting and Clinical Psychology 1983; 5(4):565-572.

Biondi M, Pancheri P. Mind and immunity. A review of methodology in human research. Advances in Psychosomatic Medicine 1987; 17:234-251.

Bonavida, B., Bradley, T.P., Grimm, E.A. Frequency determination of killer cells by a single-cell cytotoxic assay. 1983 Methods in Enzymology, 93:270-280.

Cella DF, Perry SW. Reliability and concurrent validity of three visual-analogue mood scales. Psychol Rep 1986; 59:827-833.

Claus, E.D., Risch, N., Thompson, W.D. (1994). Autosomal dominant inheritance of early-onset breast cancer. Cancer, 73: 643-651.

Delahanty, D.L., Dougall, A.L., Hawken, L., Trakowski, J.H., Schmitz, J.B., Jenkeins, F.J., & Baum, A. (1996). Time course of natural killer cell activity and lymphocyte proliferation in response to two acute stressors in healthy men. Subjects will be seen in their homes in order to reduce subjects' time commitment and increase compliance. Health Psychology, 15(1), 48-55.

Dorian BJ, Keystone E, Garfinkel PE, Brown GM. Aberrations in lymphocyte subpopulations and function during psychological stress. Clinical and Experimental Immunology 1982; 50:132-138.

Glaser R, Kiecolt-Glaser JK, Bonneau RH, Malarkey W, Kennedy S, Hughes J. Stress-induced modulation of the immune response to recombinant Hepatitis B vaccine. Psychosomatic Med 1992; 54:22-29.

Guanti G, Massari S, Cristofaro G, Caruso ML, Porsia R, Stella A, Susca F, Tauro A, Giorgio I. Depressed level of natural killer cells in cancer family syndrome. Cancer Immunol Immunother 1989; 30:307-311.

Halvorsen R, Vassend O. Effects of examination stress in some cellular immunity functions. Journal of Psychosomatic Research 1987; 31:693-701.

Harris JR, Lippman ME, Veronesi U, Willett W. Breast cancer. New England Joural of Medicine 1992; 327(5):319-328.

Herbert TB, Cohen S. Stress and immunity in humans: A meta-analytic review. Psychosomatic Medicine 1993; 55:364-379.

Hersey P, Edwards A, Honeyman M, McCarthy WH. Low natural killer-cell activity in familial melanoma patients and their relatives. Brit J Cancer 1979; 40:113-122.

Irwin M, Daniels M, Smith TL, Bloom ET, Weiner H. Impaired natural killer cell activity during bereavement. Brain, Behavior, and Immunity 1987; 1:98-104.

Kash KM, Holland JC, Halper MS, Miller DG. Psychological distress and surveillance behaviors of women with a family history of breast cancer. J Natl Cancer Inst 1992; 84:24-30.

Kiecolt-Glaser J, Dura JR, Speicher CE, Trask J, Glaser R. Spousal caregivers of dementia victims: Longitudinal changes in immunity and health. Psychosomatic Medicine 1991; 53:345-362.

Kiecolt-Glaser J, Fisher LD, Ogrocki P, et al. Marital quality, marital disruption, and immune function. Psychosomatic Medicine 1987; 49:13-34.

Kiecolt-Glaser JK, Cacioppo JT, Malarkey WB, Glaser R. Editorial Comment: Acute psychological stressors and short-term immune changes: What, why, for whom, and to what extent? Psychosomatic Medicine 1992; 54:680-685.

Kiecolt-Glaser JK, Glaser R, Dyer C, Shuttleworth E, Ogrocki P, Speicher CE. Chronic stress and immunity in family caregivers of Alzheimer's Disease victims. Psychosomatic Medicine 1987; 49:523-535.

Kiecolt-Glaser JK, Glaser R. Methodological issues in behavioral immunology research with humans. Brain, Behavior, and Immunity 1988; 2:67-78.

Kiecolt-Glaser JK, Kennedy S, Malkoff S, Fisher L, Spiecher C, Glaser R. Marital discord and immunity in males. Psychosomatic Medicine; 1988; 50:213-230.

Lotzova E. Natural killer cells: Immunobiology and clinical prospects. Cancer Investigation 1991; 9:173-184.

Manuck SB, Cohen S, Rabin BS, Muldoon MF, Bachen EA. Individual differences in cellular immune response to stress. Psychological Science 1991; 2:111-115.

McKinnon W, Weisse CS, Reynolds CP, Bowles CA, Baum A. Chronic stress, leukocyte subpopulations, and humoral response to latent viruses. Health Psychology 1989; 8(4):389-402.

McNair DM, Lorr M, Droppleman LF. Manual: Profile of Mood States. San Diego:Education and Industrial Testing Service, 1971.

Pardine P, Napoli A. Physiological reactivity and recent life-stress experience. Journal of Consulting and Clinical Psychology 1983; 3:476-469.

Pross HF, Sterns E, MacGillis DR. Natural killer cell activity in women at "high risk" for breast cancer, with and without benign breast syndrome. Int J Cancer 1984; 34:303-308.

Rees, R.C. & Platts, A.A. (1983). A modified short-term cytotoxicity test: Assessment of natural cell-mediated cytotoxicity in whole blood. Journal of Immunological Methods, 62, 79-85.

M, Jacobs R, Sttratman G, Richter S, Haedicke A, Tewes U, Wagner T, Schmidt R: Changes of natural killer cells during acute psychological stress. Journal of Clinical Immunology 1993; 13:119-126.

Schleifer SJ, Keller SE, Camerino M, Thornton JC, Stein M. Suppression of lymphocyte stimulation following bereavement. Journal of the American Medical Association 1983; 250:374-377.

Sieber WJ, Rodin J, Larson L, Ortega S, Cummings N, Levy S, Whiteside T, Herberman R: Modulation of natural killer cell activity by exposure to uncontrollable stress. Brain, Behavior, and Immunity 1992; 6: 141-156.

Stone AA, Valdimarsdottir HB, Katkin ES, Burns J, Cox DS, Lee S, Fine J, Ingle D, Bovbjerg DH. Effects of mental stressors on mitogen-induced lymphocyte responses in the laboratory. Psychology and Health 1993; 8:269-284.

Strayer DR, Carter WA, Brodsky I. Familial occurrence of breast cancer is associated with reduced natural killer cytotoxicity. Breast Cancer Research Treatment 1986; 7:187-192.

Strayer DR, Carter WA, Mayberry SD, Pequignot E, Brodsky I. Low natural cytotoxicity of peripheral blood mononuclear cells in individuals with high familial incidences of cancer. Cancer Research 1984; 44:370-374.

Trinchieri G. Biology of natural killer cells. Advances in Immunology 1990; 47:187-376.

Valdimarsdottir HB, Bovbjerg DH, Kash KM, Holland JC, Osborne MP, Miller DG. (1995). Psychological distress in women with a familial risk of breast cancer. Psycho-Oncology, 4, 133-141.

Whiteside TL, Herberman RB. The role of natural killer cells in human disease. Clinical Immunology and Immunopathology 1989; 53:1-23.

Zakowski SG, McAllister CG, Deal M, Baum A (1992). Stress, reactivity and immune function. Health Psychology, 11:223-232.

Zakowski, S.G., Cohen, L., Hall, M.H., Wollman, K., and Baum, A. (1994). Differential effects of active and passive laboratory stressors on immune function. *International Journal of Behavioral Medicine*,1, 163-184.

Zakowski, S.G., McAllister, C.G., Deal, M., and Baum, A. (1992). Stress, reactivity and immune function in healthy men. *Health Psychology*, 11, 223-232.

Zakowski, S.G., Valdimarsdottir, H.B., Bovbjerg, D.H., Borgen, P., Kash, K., Miller, D., Mitnick, J., Osborne, M., VanZee, K., & Holland, J. (In press). Predictors of intrusive thoughts and avoidance in women with family histories of breast cancer. Annals of Behavioral Medicine.

Zakowski, S.G., Valdimarsdottir, H.B., Fasano, J., Gandhi, Z., Chen, L., Waldman, G., Bovbjerg, D. (1997). Changes in mood are associated with changes in natural killer cell activity following acute laboratory stressors. *Psychosomatic Medicine, 59*, 82.

Table 1: Psychophysiological responses to the stressors in High and Normal Risk women

	Normal Risk Group	High Risk Group
Mean VAS (in mm)		
-Baseline	21.67 (9.5)	21.27 (11.6)
-Stress	39.33 (14.7)	48.56 (9.9)
Mean Heart Rate (in bpm)		
-Baseline	74.14 (9.54)	72.06 (8.21)
-Stress	84.1 (13.6)	88.03 (17.15)
Mean SBP (in mm/Hg)		
-Baseline	113.7 (8.7)	113.2 (3.6)
-Stress	129.0 (10.6)	131.8 (11.2)
Mean DBP (in mm/Hg)		
-Baseline	71.6 (5.7)	72.9 (6.7)
-Stress	83.0 (6.4)	83.2 (8.0)

Table 2: Percent change in NKCA lysis from baseline to post-stressor levels in High and Normal Risk women

	Normal Risk Group	High Risk Group
Dilution 1:1	33.40 (32.84)	63.46 (56.76)
Dilution 1:2	30.52 (29.44)	61.63 (49.87)
Dilution 1:4	19.39 (19.32)	39.98 (33.92)
Dilution 1:8	12.43 (10.91)	25.04 (25.26)

Figure 1: NKCA changes in experimentals vs. controls.